BLOOD PARASITES OF MAMMALS OF THE CALIFORNIAN SIERRA NEVADA FOOTHILLS, WITH SPECIAL REFERENCE TO *TRYPANOSOMA CRUZI* CHAGAS AND *HEPATOZOON LEPTOSOMA* SP. N.

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Introduction

Blood films were made from 509 native mammals during the summers of 1950, 1951, and 1952 at the San Joaquin Experimental Range, O'Neals, Madera Co., California, while the writer engaged in the study of the ecology of parasitic diseases, especially *Trypanosoma cruzi* Chagas. Four more samples were received in 1953, bringing the total to 513. This sampling program was greatly facilitated by concurrent studies of other investigators on the mammals and reptiles in areas where permanent drift traps were maintained. The prime purpose of this paper is to identify and enumerate the blood protozoa.

The writer is indebted to the Pacific Southwest Forest and Range Experiment Station, Berkeley, for use of facilities at the San Joaquin Experimental Range, O'Neals, California; to Dr. E. L. Biberstein, University of California, Davis, for identification of the spirochetes; to Dr. F. D. Wood for suggestions in preparation of the figures; and to Dr. G. H. Ball, University of California, Los Angeles, for supervision of and helpful suggestions in preparation of the manuscript.

Previous publications on trypanosomes in conenose bugs (Wood, 1950), contaminative effect of conenose bugs (Wood, 1951a), trypanosome development in bat bedbugs (Wood, 1951b), annoyance by house invading conenose bugs (Wood, 1951c), occurrence of blood parasites in 215 range mammals (Wood, 1952a), bug and mammal transport (Wood and Wood, 1952), natural occurrence of *Trypanosoma cruzi* (Wood, 1952b), and prevalence of ectoparasites (Augustson and Wood, 1953) have dealt with related studies in this area. Additional details of the general environment, wildlife habitat and related investigations can be found in Hutchison and Kotok (1942) and Reppert and Green (1958).

Materials and Methods

Most live animals were transported in small wire cages or live traps to the headquarters laboratory building where a fresh blood sample and one blood smear were taken from each animal. The fresh blood

was examined immediately and if spirochetes, trypanosomes, or *Plasmodium* microgametes were seen, additional smears were made.

All terrestrial mammals were marked by a standard toe clipping system of the station and released in the original area of capture. No marked land animals were recaptured. Bats were marked with aluminum numbered bands from the U. S. Fish and Wildlife Service and some individuals were recaptured at irregular intervals, usually in new roosts.

Rodents were run into wire cylinders from transport cages or live traps and blood taken from the clipped tail or ears before marking. *Microtus* and *Peromyscus* were sampled from ear veins. Dead animals were sampled from the heart in addition to tissue touch preparations from internal organs. Some samples were taken at the field plots where ecological studies were under way. Since this is a dry dusty area for most of the year, the smears show the usual contaminants of field prepared slides.

Slides were stained with Jenner-Giemsa, labelled and stored for future study. The 1950 slides were mounted in Piccolyte and all others in Grubler's Euparal Green.

Rapid examination of the summer 1950 slides revealed 30 infections. The recognition of pathological blood patterns and abnormal cells led the writer to use differential blood counts in searching for parasites in all other slides. A minimum of 200 leukocytes was counted on all slides reported here. This technique resulted in detection of many more parasites as noted below although some increase is attributable to seasonal sampling.

The per cent of host cells infected and the number of parasites for each 100 leukocytes were recorded for each differential count. Since age of infection was unknown, great variation in numbers of leukocytes was expected. Differentiation of neutrophils is the same as previously used for *Peromyscus californicus* (Wood, 1937).

Observations

One hundred twenty-five bacterial, protozoal, and helminthological blood infections were found in 513 mammals representing 19 genera and 22 species, a general infection rate of 24.3%. Thirty infections, or 13.9%, were recognized in 1950 from 215 mammals. Seventy-seven infections, or 34.1%, were detected in 225 in 1951 and 19, or 26%, were found in 73 examined in 1952 (69) and 1953 (4).

Table 1 lists the number of uninfected and infected hosts. Chroma-

Table 1

Blood parasites of mammals from the San Joaquin Experimental Range, O'Neals, California

HOSTS	Totals	Negative	Bacteria		Protozoa							Worms
			Haemobartonella	*Borrelia*	*Trypanosoma cruzi*	*Trypanosoma microti*	*Trypanosoma neotomae*	*Trypanosoma peromysci*	*Trypanosoma vespertilionis*	*Plasmodium*	*Hepatozoon*	*Microfilaria*
		♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀
Antrozous pallidus pacificus	142	58, 49		1					12, 3	10, 9		
**Eptesicus fuscus bernardinus*	11	2, 7							1			1
Microtus californicus mariposae	76	26, 16	1			11, 6					11, 10	1
Neotoma fuscipes streatori	13	4, 7					2					
Perognathus californicus ochrus	7	2	1, 4									
Perognathus inornatus inornatus	14	9, 4	1									
Peromyscus boylii boylii	48	20, 20	1	1				1, 4			1, 1	
Peromyscus maniculatus gambelii	86	43, 35									4, 3	1
Peromyscus truei gilberti	52	15, 19	2, 1	4, 1				3, 2			3, 3	
Reithrodontomys megalotis longicaudus	2	1	1		2, 1							
	TOTALS:		12	7	3	17	2	10	16	19	36	3

*Collected at North Fork, California

Table 2

Seasonal blood parasite infection summary for *Peromyscus and Microtus*

Rodent Host	Spring			Summer			Winter		
	Neg.	Pos.	%	Neg.	Pos.	%	Neg.	Pos.	%
Microtus californicus mariposae	28	11	28.2	46	20	30.3		2	100
Peromyscus boylii boylii	6			23			19	8	29.6
Peromyscus maniculatus gambelii	18	4	18.1	47			21	4	16
Peromyscus truei gilberti	9	6	40	33	10	23.2	10	1	9

tin remnants representing nuclear fragments of *Hepatozoon* or *Trypanosoma* were found in the monocytes on smears from 4 ♂ and 4 ♀ *Microtus californicus mariposae* R. Kellogg. 4 ♂ and 1 ♀ *Peromyscus boylii boylii* (Baird) and 3 ♂ *Peromyscus truei gilberti* (J. A. Allen). This means these rodents were infected at the time sampled although no intact parasites or trypanosome "shadows" could be found. The nuclear-like structure of these larger granular masses of irregular size but generally spherical shape is distinct from the occasional small azurophil granules seen in normal monocytes.

Double infections with *Hepatozoon citellicola* and *Trypanosoma microti* were found in 3 ♂ and 3 ♀ *Microtus californicus*. *Trypanosoma microti* and microfilariae were found in 1 ♀ *Microtus*. *Hepatozoon muris* with *Trypanosoma cruzi* was found in 1 ♂ *Peromyscus truei*. *Hepatozoon muris* and *Trypanosoma peromysci* were found in 1 ♂ and 1 ♀ *Peromyscus truei*. *Borrelia* and *Haemobartonella* were found in 1 ♀ and *Haemobartonella* with *Hepatozoon muris* in another ♂ *Peromyscus truei*.

Special effort was made to trap *Peromyscus* since this rodent was found previously to be susceptible to infection with *Trypanosoma cruzi* from California sources (F. D. Wood, 1934, S. F. Wood, 1937). General results for these rodents are summarized in Table 2 and indicate an infection rate of 20.4%.

Figures 1 and 2 of Plate I are the first illustrations of *Trypanosoma cruzi* from a naturally infected native host in California. The typical regressive C form blood trypanosome (Pl. I, Fig. 1) was found in a 375 sq. mm. smear from 1 ♂ *Peromyscus truei gilberti* trapped 8-IV-52 from the garage shed near the horse barn in the headquarters area. The trypanosome shows a deep basophilia characteristic of active forms resident in the blood for some time.

Slides from a previously reported *Peromyscus truei gilberti* revealed five typical blood forms in one thick drop and the one intact parasite of Pl. I, Fig. 2 (Wood, 1952b). This transitional regressive parasite shows increasing vacuolization and volutinization associated with the assumption of a rounded body form between figs. 2 and 3 of Pl. I of Wood (1953). The lighter, more dispersed basophilia is a reflection of the increased volume of the parasite preparatory to fission. The flagellum and undulating membrane begin resorption as the kinetoplast complex becomes more rounded and the parasite folds upon itself assuming the rounded leishmaniform shape. These parasites probably drift into capillary networks where, if host resistance is overcome, they complete regression to the leishmaniform stage and by cell division

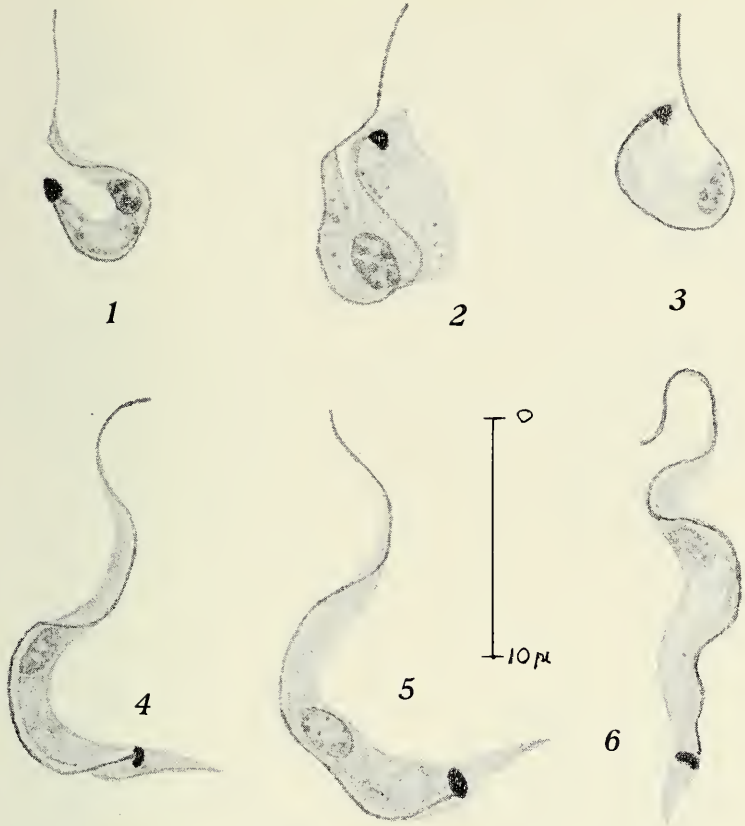


Plate I. All figures were drawn with the aid of a camera lucida. *Fig. 1.* Typical regressive C form *Trypanosoma cruzi* from ♂ *Peromyscus truei gilberti*, 8-IV-52. *Fig. 2.* Circulating regressive *Trypanosoma cruzi* from ♀ *Peromyscus truei gilberti*, 6-VII-50. *Fig. 3.* C form *Trypanosoma vespertilionis* from tissue contact lung preparation of ♂ *Antrozous pallidus pacificus*, 22-VIII-50. *Fig. 4. Trypanosoma peromysci* from ♀ *Peromyscus b. boylii*, 30-XII-51. *Fig. 5. Trypanosoma microti* from ♂ *Microtus californicus mariposae*, 26-VI-51. *Fig. 6. Trypanosoma neotomae* from ♂ *Neotoma fuscipes streatori*, 8-IV-52.

give rise to more progressively differentiating parasites which repopulate the blood stream as does *Trypanosoma zapi* (Davis, 1952). Recent studies by Rêgo (1956) report developmental forms of *Trypanosoma cruzi* in the circulating blood of white mice. This is probably the way long standing light blood parasitemias are maintained although limited foci of reticulotropic or myotropic forms are also present.

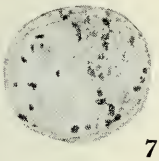
Microtus neutrophils averaged 2.7 (6.6%) segemented forms (0.5 to 7), 27.6 (68.5%) type (a) cells (8 to 53.5), 8.8 (21.7%) type (b) cells (0.5 to 23), and 1.3 (3.2%) unsegmented forms (0 to 4.5) for each 100 leukocytes from 23 infected voles. Six *Microtus californicus* revealing infections with *Hepatozoon citellicola* (Pl. II, Figs. 9 and 10) during the differential counts showed 7.1% (3.2 to 15.5) infection of monocytes. Three of these voles also revealed 81.1% (66.6 to 100) infection of unsegmented neutrophils or 14.2% (1.8 to 37.7) of all classes of neutrophils. Trypanosome and hepatozoan infections stimulate a shift to the younger type (b) and unsegmented neutrophils. In six double infections with *Trypanosoma microti* (Pl. I, Fig. 5) and *Hepatozoon*, trypanosomes averaged 111.6 (0.5 to 328.5), hepatozoans (2 infections) 0.5, one parasite in a monocyte (5.8%) and one in an unsegmented neutrophil (100%). For seven voles with *T. microti* only, trypanosomes averaged 38.7 (1 to 111.5).

Monocytes averaged 23.7% (10 to 35) for 14 infected *Peromyscus*. Neutrophils for these same mice averaged 0.6 (2.2%) segmented forms (0 to 3), 15.9 (59.3%) type (a) cells (4.5 to 43), 7.1 (26.4%) type (b) cells (1 to 21), and 3.2 (11.9%) unsegmented forms (0 to 11) for each 100 leukocytes. In one *Peromyscus boylii* with *Hepatozoon muris* (Pl. II, Fig. 11) three parasites were found in monocytes (9.6%). In four mice with *Trypanosoma peromysci* (Pl. I, Fig. 4), trypanosomes averaged 280.7 (32 to 805).

In three *Peromyscus maniculatus gambelii* (Baird), *Hepatozoon leptosoma* (Pl. II, Figs. 13 and 14) averaged 3.6 (0.5 to 8) with 6.5% (2.3 to 10) in monocytes and 45.8% in unsegmented neutrophils (two mice).

In *Peromyscus truei*, one mouse with *Hepatozoon muris* (Pl. II, Fig. 12) and *Trypanosoma cruzi* (Pl. I, Fig. 1) revealed 1.5 hepatozoans in monocytes (7.8%) and two mice with *H. muris* and *T. peromysci* revealed 0.5 hepatozoans, 0 and 1, in monocytes (6.2%) and 8.7 trypanosomes, 0 and 17.5. Two mice with *T. peromysci* only averaged 24.2 trypanosomes (0 and 48.5) for each 100 leukocytes.

The following mammals were negative for blood parasites: 2 ♂, 1 ♀ *Spermophilus beecheyi fisheri*, 14 *Dipodomys heermanni tularensis*, 1 ♂ *Eutamias m. merriami*, 1 ♂, 1 ♀ *Lepus c. californicus*, 1 ♂ *Lynx rufus californicus*, 2 ♂, 4 ♀ *Myotis yumanensis sociabilis*, 1 ♀ *Pipistrellus hesperus merriami*, 1 ♂, 2 ♀ *Procyon lotor psora*, 1 ♀ *Scapanus l. latimanus*, 1 ♂, 7 ♀ *Sylvilagus audubonii vallicola*, 2 ♂ *Tadarida brasiliensis mexicana*, and 13 ♂, 7 ♀ *Thomomys bottae mewa*.



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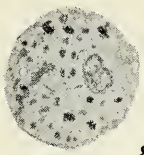

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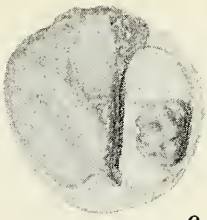

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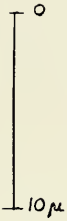

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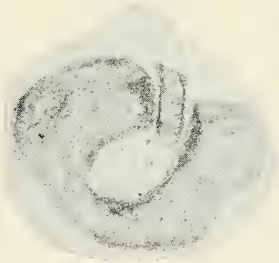

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Plate II. Fig. 7. Microgametocyte of *Plasmodium* sp. from ♂ *Antrozous pallidus pacificus*, 9-VII-51. *Fig. 8.* Macrogametocyte of *Plasmodium* sp. from ♂ *Antrozous pallidus pacificus*, 9-VII-51. *Fig. 9. Hepatozoon citellicola* in monocyte from ♂ *Microtus californicus mariposae*, 19-IV-51. *Fig. 10. Hepatozoon citellicola* in unsegmented neutrophil from ♂ *Microtus californicus mariposae*, 19-IV-51. *Fig. 11. Hepatozoon muris* in a monocyte from ♂ *Peromyscus b. boylii*, 30-XII-51. *Fig. 12. Hepatozoon muris* from a monocyte from ♂ *Peromyscus truei gilberti*, 8-IV-52. *Fig. 13. Hepatozoon leptosoma* sp. n., freed by rupture from a monocyte from ♀ *Peromyscus maniculatus gambelii*, 8-IV-52. *Fig. 14. Hepatozoon leptosoma* sp. n., in a monocyte from ♀ *Peromyscus maniculatus gambelii*, 8-IV-52.

Hepatozoon citellicola (Wellman and Wherry, 1910)

Gametocytes (Pl. II, Figs. 9 and 10) on one stained slide from a California vole, *Microtus californicus*, averaged 9.3 μ long (8.1 to 10.3) by 3.9 μ wide (3.3 to 4.5) for 25 specimens chosen at random. The gametocyte nuclei of these parasites averaged 4.2 μ long (3.9 to 5.1) by 3.3 μ wide (3 to 3.9).

This sausage-shaped parasite exhibits a distinct limiting plasma membrane which pulls away from the host cell cytoplasm in some cells. The lightly basophilic cytoplasm appears irregularly vacuolated towards each end of the cytosome. The average number of metachromatic granules was 9.8 (6 to 14) for the 25 parasites. These are similar to volutin granules of trypanosomes and occur in the nuclear area or scattered through the cytoplasm. Most parasites lie parallel to the main axis of orientation of the monocyte nucleus but occasionally the organism lies within the crescentic cavity of the horseshoe shaped structure.

The parasite nucleus is irregularly oval or rounded in shape tending to be slightly elongate. The peripheral chromatin (basichromatin) appears in irregular clumps or bands separated by compact or broad irregular parachromatin (oxychromatin) spaces and surrounding a less dense central core of nucleoplasm.

Hepatozoon citellicolla is parasitic in the monocytes and unsegmented neutrophils of the blood of *Microtus californicus mariposae*, San Joaquin Experimental Range, O'Neals, California. There is no way at present of distinguishing this parasite from that described by Wellman and Wherry (1910) from the California ground squirrel, *Spermophilus beecheyi*. Since the same species of ground squirrel is commonly associated with the foothill mammals studied here, their name is used in preference to *Hepatozoon microti* (Coles, 1914) until more data is available on the life cycle.

The host cells of the blood of the vole are the unsegmented neutrophils and monocytes. The cytoplasm of the unsegmented neutrophil is moderately basophilic, lightly vacuolated, and with none or a few distinct neutrophilic granules.

The ring shaped neutrophil nucleus shows a compact chromatin structure similar to the smaller monocytes and larger lymphocytes. Where the central aperture has enlarged and the circular, band-form nucleus has become more labile, the parasite may extend into the immature neutrophils have not yet developed their distinctive granulation. Older cells with more irregular banded nuclei show the diag-

nostic granules clearly. One segmented type (a) neutrophil showed *Hepatozoon* remnants in the densely granulated cytoplasm similar to those seen in monocytes.

The moderately basophilic monocyte cytoplasm has a lightly vacuolated and granular appearance. Granules are fine or coarse. The finely granular appearance is due to the presence of normal azurophil spherules similar to that found in the blood of other rodents. The coarse granules, numbering 22 to 27 in the cells counted, are breakdown products of the nuclei of hepatozoans or trypanosomes as shown by the similar structure and staining in the nuclei of these parasites. The general absence of a perinuclear halo, variable shaped nuclear structure, and extensive less basophilic cytoplasm distinguish this cell from the lymphocyte. There are medium sized lymphocytes in *Microtus* blood with looser chromatin organization suggesting a transition form to the monocyte.

The monocyte nucleus has broad or narrow dense chromatin masses with narrow or broad irregular parachromatin spaces. In younger cells it is rounded or slightly crescentic. Older parasitized cells show an elongate C shaped structure in which occasionally the lobes are folded back over each other. The labile nucleus in one instance was folded S like along one side of the cytosome.

Specimens are deposited at the Department of Zoology, University of California, Los Angeles.

Hepatozoon muris (Balfour, 1905)

Gametocytes (Pl. II, Figs. 11 and 12) on one slide from Boyle's white-footed mouse, *Peromyscus boylii*, averaged 8.2 μ long (7.8 to 9.3) by 3.5 μ wide (3 to 4.2) for 15 specimens chosen at random. The gametocyte nuclei of these parasites averaged 3.5 μ long (2.7 to 4.2) by 2.8 μ wide (2.1 to 3.3). Measurement of seven parasites from four slides of the Gilbert white-footed mouse, *Peromyscus truei*, averaged 8 μ long by 3.4 μ wide for the cytosome and 4 μ by 3.1 μ for the nuclei.

Terminal cytoplasmic caps within the parasites' plasma membrane of a substance staining similarly to the protoplasmic metachromatic granules or nuclear chromatin distinguished this parasite from all others studied here. The slightly shorter and broader cytosome occasionally reveals from two to six free metachromatic granules. The similarity in staining reaction of the substance of the cytoplasmic caps to that of the metachromatic granules and nuclear chromatin suggests

origin from these cytosomal components. In some parasites, the caps form a distinct broad band, 1 to 2 u thick at the center, and tapering away to the plasma membrane halfway from the end of the cytosome to the edge of the central nucleus. Parasites freed by rupture of the host cell and those extending into the host cell protoplasm without overlying the nucleus show this structure most clearly. Although appearing homogeneous in side view in some cells, it is more granular appearing in others. This narrower parasite appears more often in varying positions in the host cell. It was found along the convex surface of the host nucleus, within the elongate U shaped lobes, or extending across the cytosome at an angle to the nucleus.

The parasite nucleus is oval or rectangular with rounded corners. The chromatin is peripherally concentrated in broad bands with irregularly shaped less extensive parachromatin spaces. Some nuclei appear nearly solid from one surface.

Hepatozoon muris is parasitic in the monocytes of the blood of *Peromyscus boylii boylii* and *P. truei gilberti* from the San Joaquin Experimental Range, O'Neals, California. This parasite is probably the same as the parasite described by Balfour (1905) from mononuclear leukocytes of *Mus decumanus* and whose life history was reported by Miller (1908). Although the cytoplasmic caps were indicated by Balfour (1905), Porter (1908) and Mackerras (1959), they were not mentioned by Miller (1908). Hoogstraal (1957) noted these "caps" in *Hepatozoon balfouri*.

The monocyte cytosome exhibits a lightly basophilic reticulated cytoplasm often suggesting an alveolar pattern. There is sometimes a perinuclear halo.

The monocyte nucleus shows broad homogenous, dense basichromatin masses separated with a few irregular, parachromatin spaces detracting little from the general homogeneity of the structure. The oval or slightly indented nucleus of normal cells becomes U shaped in response to parasitism.

Specimens are deposited at the Department of Zoology, University of California, Los Angeles.

Diagnosis

Hepatozoon leptosoma, new species

Gametocytes (Pl. II, Figs. 13 and 14) on one slide from a Gambel white-footed mouse, *Peromyscus maniculatus*, averaged 10.5 μ long (9.6 to 11.5) by 2.1 μ wide (1.8 to 2.7) for 15 specimens chosen at

random. The gametocyte nuclei of these parasites averaged 4.6 μ long (2.4 to 6) by 1.7 μ wide (1.5 to 2.4).

The elongated, crescent shaped parasite often occupies a position within the concavity of the horseshoe-shaped nucleus of the monocyte (Pl. II, Fig. 14). It is sharply delimited by a distinctly stiff plasma membrane. It is narrowly elongate and crescentic with slightly basophilic, more homogeneous cytoplasm containing from five to twelve metachromatic granules in some specimens. Although the commonest orientation of the parasites is enclosed in the arms of the U shaped nucleus of monocytes, some parasites lie under the plasma membrane of the unsegmented neutrophils on the concave or convex side of the host cell nucleus. In one instance, a parasite extended through the opening of the neutrophil nucleus. In some parasites with terminally displaced nuclei, a clearer large vacuolated area occupied the opposite end of the cytosome.

In surface view, the oblong parasite nucleus consists of broad chromatin bands with narrow irregular parachromatin spaces (Pl. II, Fig. 13). In some nuclei the bulk of the chromatin was distributed peripherally presenting a tube effect with a central nucleoplasmic core. In other parasites, the nuclear chromatin appeared barrel shaped with parachromatin scattered through the nucleoplasmic core. In one instance, the chromatin and parachromatin mass appeared cup-shaped.

Hepatozoon leptosoma is parasitic in the monocytes and unsegmented neutrophils of the blood of *Peromyscus maniculatus gambelii* at the San Joaquin Experimental Range, O'Neals, California.

The host monocyte is similar to those described for *Peromyscus californicus insignis* (Wood, 1937). Most nuclei of monocytes have an elongate C shape enclosing the parasite. There are also numbers of unsegmented neutrophils with heavy ring shaped nuclei of broad basichromatin bands and narrow oxychromatin spaces crowding the parasite to the edge of the cytosome.

Hepatozoon leptosoma may be distinguished from other California rodent hepatozoans by size, narrow elongate form and preferred host.

Type specimens are deposited in the Department of Zoology, University of California, Los Angeles.

Xenodiagnosis

Xenodiagnosis was carried out for 186 mammals in 1950, 99 in 1951 and 71 in 1952 and 1953. The 356 mammals included 253 rodents, 91 bats, 8 rabbits, 2 raccoons and 2 domestic cats. This involved the feeding of 1,275 conenose bugs, mostly laboratory raised, including

896 *Triatoma p. protracta* (Uhler), 321 *T. rubida uhleri* (Neiva), 49 *Paratriatoma hirsuta* Barber, and 9 *Triatoma recurva (longipes)* Stål. Most of these bugs were 1st and 2nd instar nymphs at time of initial blood meal.

The 12 infections in *Triatoma p. protracta* were from three Gilbert white-footed mice, *Peromyscus truei gilberti*, from the range headquarters area. Six 1st instar nymphs fed on 1 ♀ trapped 6-VII-50 in an old wood pile, three of four 1st fed on 1 ♂ trapped 13-VII-50 near buildings, and three 2nd fed on 1 ♂ trapped 8-IV-52 from the open garage shed near the horse barn showed metacyclic *Trypanosoma cruzi* in voluntary fecal droplets released from the 94th to 104th days after the original infective meal.

Discussion

Miller (1908) used the term mononuclear for lymphocytes of the rat but listed the infected cells as large lymphocytes. Harris (1960) points out that only monocytes and neutrophils are phagocytic. Miller (1908) states that *Hepatozoon* is engulfed by the host cell with no effect on it but Balfour (1905), Porter (1908) and Laird (1959) infer active penetration of the host cell or nucleus by the parasite. If *Hepatozoon* actively penetrates the host cell, it could be found in any adaptable blood cell. If we follow Harris (1960) and Miller (1908) as to engulfment, then the host cells are neutrophils or monocytes. *Hepatozoon canis* is reported from neutrophils (Laird, 1959). If the *Hepatozoon* gametocyte is mature when released by the internal tissue cells, engulfment would seem logical. In some species the red blood corpuscles are invaded and "young" gametocytes are found so this would indicate active penetration (Hoogstraal, 1957, Wellman and Wherry, 1910). In heavy *Hepatozoon citellicola* infections of *Microtus*, both monocytes and neutrophils contain parasites but not the lymphocytes. Coles (1914) reported karyolytic action on the monocyte nucleus in *Hepatozoon microti* which was not noted here. The above observations suggest engulfment of *Hepatozoon citellicola* by the host cells in view of known function (Harris, 1960). Research on the cellular reactions to *Hepatozoon* might add new insight to the functions of leukocytes.

Analysis of the data as to season reflects the relationship of environmental temperature to intensity of blood parasitemias. This is a definite clue to use for retrieving material for additional study with the exception of those mammals restricted to warmer activity periods as bats. Summer samples numbered 378, spring 76, and winter 59 with 74, 23, and 20 infections, respectively, for rates of 19.5, 31.2 and 33.8

per cent. If we deduct the bats from the summer total, the infection rate drops to 17.1%.

Of the 162 bats examined, 37 or 22.8% were infected. These mammals offer a special study of the relationship of *Trypanosoma cruzi* and *T. vespertilionis* which are closely allied species as shown in Pl. I, Figs. 1 and 3. The life cycle of the *Plasmodium* (Pl. II, Figs. 7 and 8) from *Antrozous* deserves intensive study since this bat can be maintained in captivity.

The use of the differential leukocyte count is well known as a diagnostic aid in clinical hematology. It was used here as an aid in enumerating and finding blood parasites, and to standardize coverage of the smear. The same problem exists here for differentiation of lymphocytes and monocytes as occurs in human blood (Fey, 1958, Harris, 1960). Until detailed studies of normal blood of native rodents are made, it is not possible to relate accurately changes in the blood picture to infection. Therefore, the writer has used normal figures for *Peromyscus californicus* and *Mus musculus* for the comparisons indicated below.

The structure of the rodent monocyte host cell is similar to that of human blood in size of the cytosome, extent and staining of the cytoplasm and the usual absence of a perinuclear halo. The dense banded and blotched pattern of basichromatin with little oxychromatin is more distinctive of lymphocyte nuclei than monocytes where the amount of basichromatin and oxychromatin is more equal. The extensive cytoplasm of these leukocytes of *Peromyscus* and *Microtus* and the variable nuclear shape in an irregular S, C or U form agrees with the differentiation for white mice (Fey, 1958). There is also considerable numerical variability in normal white mice (Snell, 1941). Detailed study of monocytes and lymphocytes led to the recognition of the parasite remnants from normal cytoplasmic granulation. Both hepatozoans and trypanosomes contribute to these remnants. Similar chromatin remnants have been reported in polymorphonuclear neutrophils for *Hepatozoon canis* (Laird, 1959).

Differential leukocyte counts on 12 normal *Peromyscus californicus* revealed 27% neutrophils, 7% eosinophils, 3% basophils, 9% monocytes, and 54% lymphocytes since rodent lymphoid cells with wide zones of cytoplasm are now considered monocytes (Fey, 1958, Harris, 1960, Wood, 1937). Normal differential counts for white mice of various strains averaged 18% neutrophils, 2% eosinophils, 11% monocytes, and 69% lymphocytes (Snell, 1941). Niño (1929) studying the blood picture of white mice infected with virulent *Trypanosoma cruzi* found an initial lymphocytosis followed by a neutrophil leuko-

cytosis 25 to 35 days after infection. The high neutrophil count then persisted in numerical balance with the lymphocyte count until death. Since Niño did not separate monocytes and the age of the wild infections here is unknown, it is not possible to compare the two sets of data.

Tabulated differential leukocyte counts here revealed a monocytosis with marked reduction of neutrophils for three *Peromyscus boylii* with *Trypanosoma peromysci* and four *P. truei*, one with *T. cruzi*, two with *T. peromysci*, and one with *T. peryomysci* and *Hepatozoon muris*. A monocytosis only was found in one *P. boylii* with *Hepatozoon muris* and one *P. truei* with *T. cruzi*. A neutrophil leukocytosis was found in one *P. maniculatus* with *Hepatozoon leptosoma* and two *P. truei*, one with *T. cruzi* and one with *T. peromysci* but both also harboring *Hepatozoon muris*.

Differential counts for the California vole, *Microtus*, revealed a lymphocytosis with reduced neutrophils in one animal with *Trypanosoma microti*. A monocytosis only occurred in three voles with *T. microti* and one with *Hepatozoon citellicola*. A monocytosis with reduced neutrophils was found in one vole with *T. microti* and two with *T. microti* and *H. citellicola*. A neutrophil leukocytosis was found in two voles with *T. microti*, seven with *H. citellicola* and two with both infections.

Galliard *et al.* (1959) demonstrated persistent low grade parasitemias in white mice with chronic infections of *Trypanosoma cruzi* long after experimental infection of the same mice with *Borrelia duttoni* and *B. crociduri*. Therefore, the presence of spirochetes in the blood of *Peromyscus truei* is probably an important factor in maintenance of *T. cruzi* infections in native rodents of California.

Summary

Blood infections of mammals with *Haemobartonella* (12), *Borrelia* (7), *Trypanosoma cruzi* (3), *T. microti* (17), *T. neotomae* (2), *T. peromysci* (10), *T. vespertilionis* (16), *Plasmodium* (19), *Hepatozoon* (36) and microfilaria (3) are recorded from central California. *Hepatozoon citellicola* is reported from *Microtus californicus mariposae* and *Hepatozoon muris* from *Peromyscus boylii boylii* and *Peromyscus truei gilberti*. *Hepatozoon leptosoma* sp. n. is described from *Peromyscus maniculatus gambelii*.

Differential leukocyte counts used in finding parasites revealed a neutrophil leukocytosis, lymphocytosis and monocytosis in infections with trypanosomes and hepatozoans.

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